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REVIEW

The chondrodystrophic dog: A clinically relevant intermediate-sized animal model for the study of intervertebral disc-associated spinal pain

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Low back pain (LBP) is the leading cause of disability worldwide, with an estimated 80% of the American population suffering from a painful back condition at some point during their lives. The most common cause of LBP is intervertebral disc (IVD) degeneration (IVDD), a condition that can be difficult to treat, either surgically or medically, with current available therapies. Thus, understanding the pathological mechanisms of IVDD and developing novel treatments are critical for improving outcome and quality of life in people living with LBP. While experimental animal models provide valuable mechanistic insight, each model has limitations that complicate translation to the clinical setting. This review focuses on the chondrodystrophic canine clinical model of IVDD as a promising model to assess IVD-associated spinal pain and translational therapeutic strategies for LBP. The canine IVD, while smaller in size than human, goat, ovine, and bovine IVDs, is larger than most other small animal IVDD models and undergoes maturational changes similar to those of the human IVD. Furthermore, both dogs and humans develop painful IVDD as a spontaneous process, resulting in similar characteristic pathologies and clinical signs. Future exploration of the canine model as a model of IVD-associated spinal pain and biological treatments using the canine clinical model will further demonstrate its translational capabilities with the added ethical benefit of treating an existing veterinary patient population with IVDD.

KEYWORDS

degeneration, pain, preclinical models

1 | INTRODUCTION

The Global Burden of Disease study of 2015 identified lower back pain (LBP) as the leading cause of disability worldwide.[1] Indeed, it is estimated that 80% of Americans will suffer from a painful back condition at some point during their lives.[1] The most common cause is degeneration of the intervertebral disc (IVD), with a prevalence of 39%-42% in LBP patients.[2] IVD degeneration (IVDD) can occur as a natural part of the aging process, or as a consequence to cell-mediated responses to structural failure.[3,4] Medical management of LBP is primarily focused on the treatment of symptomatic pain, which may alleviate clinical signs but does not restore IVD function. Surgical

management strategies can address structural problems with the disc, but have failure rates as high as 40% due to incomplete decompression, spinal instability, alteration of the vertebral column's biomechanics, epidural scar tissue formation or iatrogenic nerve injury.[5,6] Thus, there is a critical need to investigate the pathophysiology of painful IVDD and resultant LBP in order to develop safe, successful and less invasive clinical treatments.

Experimental animal models are vital for understanding the mechanisms of painful IVDD and for the early stages of developing therapeutic strategies; however, these models have some limitations that may impact their predictive value when translating an intervention from the laboratory to the clinical setting. Of recent interest from

the translation perspective is the canine "chondrodystrophic" (CD) model of IVDD, which is a spontaneous clinical model of IVDD occurring in pet dogs. IVDD affects upwards of 20% of certain breeds of dogs such as the miniature dachshund.[7] In North America alone, between 20,000 and 30,000 cases of spontaneously occurring IVDD in pet dogs are managed by veterinary spinal specialists each year.[8] As a result, the canine IVDD population is highly amenable to large scale veterinary clinical studies which can be conducted with adherence to CONSORT guidelines, National Institutes of Health (NIH) standards for scientific transparency and rigor, and can closely recapitulate a human clinical trial condition.[9,10] Of significant note, unlike most other animal models, dogs with spontaneous IVDD commonly present to the veterinary clinic with IVDD-associated pain.[11] Degeneration of the canine IVD also occurs spontaneously, making pathologic processes associated with IVDD in this species highly relevant to the human condition. Additionally, the size of a typical dog with spontaneous IVDD addresses many "scaling up" issues encountered in the direct translation of therapies from rodents to humans. Lastly, the use of pet dogs with spontaneous IVDD can contribute to reduction, refinement, and replacement of experimental animal IVD models (3R principles) by providing an ethically responsible source of nucleus pulposus (NP) material collected during surgical discectomy and cells, tissues or whole discs collected during autopsy with consent from owners of IVDD-affected dogs. For all of these reasons, there has been a recent increase in the interest of using spontaneously occurring IVDD in pet dogs as a unique model through which to conduct properly designed veterinary preclinical studies for IVDD treatment prior to entering the human clinical setting.

This narrative review focuses on models of painful IVDD with an emphasis on the utility of the CD canine clinical model of IVDD as a potential translational research tool for studies investigating IVD-associated spinal pain. It compares the major structural, cell, and tissue level characteristics across species in both health and disease together with pain-associated behavior. Lastly, it reviews current treatment strategies that have utilized cells, tissue, and whole IVDs from CD dogs and outlines areas of opportunity for further exploration of the clinical canine model of painful IVDD to maximize its value as a translational model.

2 | THE HEALTHY IVD

The general structure of the IVD is well-conserved across vertebrate species, and serves to counteract the compressive forces of the body that are placed upon the vertebral column while also acting as joints to promote motion and flexibility.[12] Under healthy conditions, the IVD is composed of the annulus fibrosus (AF), which consists of concentric lamellae of collagen type I, elastin, and fibroblast-like cells, and encloses the proteoglycan-rich NP.[13] The NP and AF are formed from the embryonic notochord and surrounding mesenchyme, respectively, during development of the IVD.[13,14] The dense collagen lamellar structure of the AF provides tensile strength and stability to the vertebral column by contributing to overall rigidity.[15] The NP is a hydrated mixture of cells, aggrecan and collagen type II[16]. Aggrecan helps to imbibe water, creating a gelatinous NP core that distributes load across the disc and absorbs compressive forces.[17] The

transitional zone (TZ) or inner AF region separates the AF from the NP in the mature IVD.[18] The IVD is avascular, relying on the permeability of the adjacent cartilaginous endplates (CEPs) to receive nutrition.[19–22] The CEP is a layer of hyaline cartilage covering the vertebral bodies that isolates the disc from the rest of the vertebral column.[22] In addition to being avascular, the healthy IVD is aneural, and can be viewed as a site of immune-privilege.[11]

3 | DEGENERATION OF THE INTERVERTEBRAL DISC

3.1 | Growth and maturation

Figure 1 demonstrates maturation of the IVD, from the young IVD (Figure 1A) through the early (Figure 1B) and late stages of IVDD (Figure 1C-E). Many species such as rodents, pigs, rabbits and non-CD (NCD) dogs have IVDs that retain notochordal cells throughout adulthood.[11,23] However, during growth or adolescence, the notochordal cells within the NP of the human and CD canine IVD, as well as intermediate and large sized animals such as sheep, cattle, and goat are replaced with chondrocyte-like cells (CLCs)[24,25] as seen in the gelatinous and less translucent NP in Figure 1B. CD dogs typically lose their notochordal cells as they reach skeletal maturity, usually within the first year of life, while humans lose theirs by approximately 10 years of age.[26] Since notochordal cells protect the NP from degradation and apoptosis, their loss is associated with an imbalance in matrix turnover and catabolism of the IVD.[27] In contrast, rodents, rabbits and most NCD dogs retain a population of notochordal cells within the IVD and thus maintain a healthy balance of matrix turnover, resulting in preservation of the structural integrity and function of the IVD.[27] While there is an association between a loss of notochordal cells and disc degeneration, this has not been unequivocally established and there are likely additional factors (ie, genetics, lifestyle, comorbidities) that are involved.

3.2 | Mild changes

Proteoglycan content of the NP is maximized during young adulthood and begins a slow and steady decline shortly thereafter due to increased fragmentation and simultaneous increase in collagen content.[20,27] The demarcation between the NP and AF becomes less apparent as the collagen fibrils of the AF encroach upon the NP (Figure 1C). During this transition, the overall cellularity of the IVD declines and the disc becomes more fibrous with increased collagen deposition.[28]

3.3 | Moderate and late-stage changes

The avascular nature of the IVD causes it to lack the innate ability to appropriately heal and repair.[11,21] Changes in the NP as a result of maturation affect the biomechanics of the IVD and ultimately lead to "wear and tear," whereby continued strenuous activities enhance matrix degradation over synthesis.[29] This inability to counteract compressive forces places additional strain on the AF, leads to AF degeneration including cleft and crack formation, and reduces its contributions to the overall strength of the vertebral column (Figure 1D).[17] Aging also leads to calcification of the CEP, which reduces porosity within the CEP
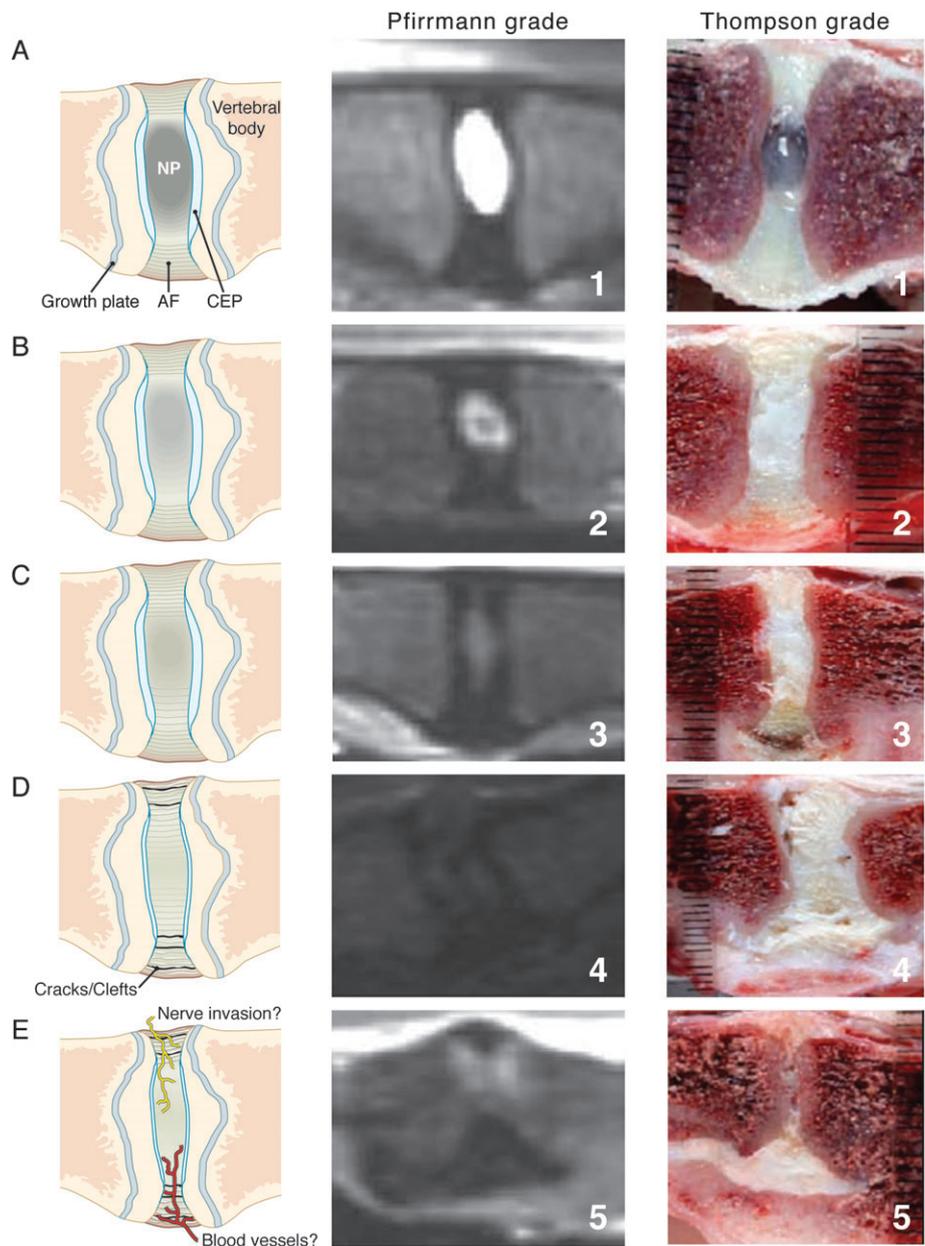
FIGURE 1 Intervertebral disc (IVD) maturation from young to early and late stage IVDD where the first column shows illustrative representations throughout the stages (A-E), middle column shows Pfirrmann grading via IVD magnetic resonance imaging (MRI) and last column showing Thompson grading of canine IVD. AF, annulus fibrosus; CEP, cartilaginous end plate; NP, nucleus pulposus. Pfirrmann grade and Thompson grade images adapted with permission from Bergknut et al. American Journal of Veterinary Research, 2011;72:899

structure itself and decreases disc access to nearby blood vessels and nutrition, enhancing a decline in cellularity. Lactic acid accumulation alters the cellular microenvironment within the disc, slowing matrix production but not matrix enzyme activity.[30]

Upregulation of inflammatory and catabolic responses in degeneration can lead to increased expression of vascular endothelial growth factor (VEGF) and nerve growth factor (NGF), resulting in the invasion of nerves and blood vessels (Figure 1E).[31] These changes in disc environment lead to changes in structure function as well as contribute to pain mechanisms.[11,32]

4 | EXPERIMENTAL ANIMAL MODELS OF IVDD

Experimental models have provided valuable insight into the mechanisms underlying disc degeneration as well as the ability to assess therapeutic interventions. Popular experimental animal models of IVDD include the mouse, rabbit, rat, pig, sheep, goat, and cattle.[11,23,33] While these models have contributed to the current understanding of IVDD pathophysiology, each presents a unique set of limitations that hinder the translation of experimental results to the human condition. Unlike the human IVD where notochordal cells are replaced during adolescence, mice, rabbits, and pigs retain their supply of notochordal cells through adulthood, while cows and sheep lose theirs rapidly after birth.[34-36] These fundamental developmental differences mean that IVDD does not typically occur spontaneously and must be artificially induced through injury.[11] Common artificial IVD injury models include annular stab, puncture, chemical or mechanical induction, altered nutrient supply, or genetic modification.[23,37] The physiological changes following artificial induction may differ from the spontaneous process occurring in people.[11] An exception to this is the spontaneous occurrence of IVDD in the sand rat; however, its small IVD size relative to the human IVD presents both logistical challenges and translational difficulties.[33]

TABLE 1 Experimental animal models of LBP in comparison with human IVDs

Animal models of LBP	Experimental models								
Characteristics of the human IVD in healthy and diseased	CD Canine[11,30,38,39]	NCD Canine[11,30,39,40]	Rodents[23,27,34,41-43]	Rabbits[27,37,41]	Cattle[37,41]	Pig[35,41]	Sand rat[33,44,45]	Goat[37,46]	Sheep[36,47]
Healthy IVD									
Hydrated NP	X	X	X	X	X	X	X	X	X
Fibrous AF	X	X	X	X	X	X	X	X	X
Large notochordal cells		X	X	X		X			
Avascular/aneural	X	X	X	X	X	X	X	X	X
Degenerate IVD									
Occurs spontaneously	X	X					X		
Matrix degradation	X	X	X	X	X	X	X	X	X
Decreased number of cells	X	X	X	X	X	X	X	X	X
Dimension reduction (disc height)	X	X	X	X	X	X		X	X
Nerve ingrowth			X						
Loss of nutrition	X	X	X	X	X	X	X	X	X
Pain	X	X	X				X		
Increased axial loading	X	X	X	X	X	X	X	X	X
Inflammation	X	X	X	X	X	X	X	X	X
Immune infiltration	-	-	X	X	X	X	X	X	X
Other characteristics									
Intermediate-large size	X	X			X	X		X	X
Clinical patient model	X	X							

Thus, in seeking out an animal model of IVDD relevant to studying the human condition, there are a number of aspects that must be considered. While the use of bipedal animals may better mimic the biomechanics of the human IVD, research with nonhuman primates is ethically challenging and often cost prohibitive; as a result, the use of quadrupeds is much more practical. However, cows, pigs, goat, and sheep IVDs have limited motion capabilities and flexibility compared to that of the human IVD.[36] Animal and IVD size is also important for the translation of pathology results and calculations for therapeutic administration. Intermediate or large sized animals are often preferable, as they minimize anatomical differences as well as errors associated with scaling up of doses for use in clinical studies. Lastly, translational IVDD models should ideally offer the ability to assess and measure pain resulting from degenerative processes. While rodents are useful for pain assessment, they do not meet the previously stated criteria, and pain cannot be adequately measured in the rabbit, cow or pig models.

Table 1 summarizes the criteria necessary for modeling human IVDD with a comparison of animal models discussed in this review. As seen, the CD canine model most closely matches the human condition for reasons to be discussed in the following section. Characteristics that are consistent across species of all sizes in the healthy IVD include the avascular and aneural nature, hydrated NP, and fibrous AF. For the degenerate IVD these include matrix degradation, decreases in cellularity, alterations in disc height, inflammation, loss of nutrition, and increased axial loading. Differences between species center largely around the presence or absence of notochordal cells through adulthood, whether disease occurs spontaneously or must be induced, and whether validated objective assessments of pain exist. Large gaps include the assessment of neurovascular ingrowth as a mechanism of pain and limited number of spontaneous intermediate to large-sized animal models to study pain in the degenerate IVD.

5 | THE CANINE CLINICAL MODEL OF IVDD

5.1 | Hansen type I and type II

IVDD-associated pain and neurologic dysfunction are a common clinical problem in pet dogs.[8] Spontaneous canine IVDD occurs in what has been classically believed to be two clinically distinct forms, described by Hansen as type I and type II IVDD.[48] Hansen type I IVDD is described as dehydration, degeneration and dystrophic calcification of the NP of the IVD, and is most commonly observed in CD dog breeds such as the dachshund, beagle, shih tzu, lhasa apso, and Pekingese. Within these breeds, the supply of notochordal cells within the NP matrix is replaced with CLCs, often as early as 2 months of age.[48,49] Typically, CD dogs display clinical signs of IVDD between 3 and 7 years of age, when dehydration of the NP places additional stress on the dorsal AF leading to rupture and extrusion of NP matrix into the vertebral canal. The IVDs of the cervical or thoracolumbar spine are the most commonly affected sites.[48,49] Dachshunds are the breed most commonly affected with type I IVDD, with a breed prevalence as high as 20%.[7]

IVDD-like pathologies in NCD dogs are associated more with age-related changes, as notochordal cells are typically retained within the NP throughout adulthood. However, certain large breed dogs such as the German shepherd and Labrador retriever may develop what has been termed Hansen type II IVDD later in life.[40,50,51] This tends to occur in regions of the vertebral column susceptible to "wear and tear" such as the caudal lumbar and lumbosacral regions.[11] Specifically, the oblique angle created by the joint of the seventh lumbosacral vertebrae and first sacral vertebra is subject to a larger amount of workload compared to the other areas of the spine, which makes it especially vulnerable.[50] Hansen type II IVDD has been classically described as fibroid degeneration and dorsal thickening of the AF; however, recent work suggests that the term chondroid metaplasia may be more appropriate to describe these pathologic changes.[38,48,52] Specifically, IVDD involves replacement of notochordal cells of the NP by CLCs in both CD and NCD species. Increased collagen content of the NP causes AF fibers to split, allowing plasma and fluid from the NP to accumulate between the AF fibers. Over time, the AF thickens due to a buildup of pressure in the area and bulges dorsally into the vertebral canal, causing spinal cord or nerve root compression.[40,50,51] Figure 2 depicts the discussed differences in the two types of Hansen herniation in canine IVDs.

IVDD by either mechanism can result in significant neurologic manifestations, including neck or back pain, radiculopathy, and in severe cases, paralysis.[53] It should be noted that while severe neurologic abnormalities can result, particularly from Hansen type I IVDD in approximately 10%-15% of cases, a substantial portion of dogs with IVDD present with spinal pain as the only clinical sign of IVDD.[54] While both canine spontaneous models of IVDD have potential value, Hansen type I IVDD in CD dogs may have the most direct relevance to the human condition. The pathophysiology is most similar to IVDD that occurs in people as changes are not just age-related but can be described as a "cell-mediated response to structural failure."[4] For this reason, the remainder of this review will focus on Hansen type I IVDD (referred to as IVDD from here on).

6 | CLINICAL RELEVANCE TO IVDD AND IVD-ASSOCIATED SPINAL PAIN

The canine model of IVDD complements experimental models by offering confirmation of promising laboratory findings in a spontaneous model of disease. Beyond its value as a confirmatory tool, the model offers additional distinct advantages. This spontaneous model of IVDD has a high degree of biologic relevance because its onset, related to the upregulation of degradation pathways within the IVD, is similar to the pathology of human IVDD.[11,38,39] The depletion of notochordal cells in CD dog IVDs during growth, replacement with CLCs, and subsequent increase in fibrous content provide for a similar NP composition to the mature human IVD. As a result, both the canine and human IVDs undergo similar pathophysiological changes during degeneration.[11,38,39]

A small number of studies have begun characterizing disc degeneration in the canine model, including comparisons between the CD and NCD breeds as shown in Table 2. While these studies characterize the degenerate process in NCD and CD species they do not highlight the similarities and differences in painful pathologies between the two. Both dogs and people diagnosed with IVDD present with clinical signs or symptoms of pain. In people, LBP is often debilitating, and can occur with or without concurrent neurological deficits. Either scenario results in a substantial impact on quality of life. CD pet dogs with IVDD are frequently presented to veterinary specialists with clinical signs of spinal pain (with or without neurologic deficits) that disrupts daily activities. This similarity offers an important advantage with respect to clinical relevance of this model.[31,48]

Because pet dogs with spontaneous IVDD represent a clinical population, they offer the ability to pursue longitudinal studies of biologic changes associated with aging and degeneration of the IVD, as well as interventional preclinical studies examining biological therapies in a genetically and environmentally diverse population that closely mirrors a human clinical trial setting.[30] Dogs with IVDD are typically diagnosed and managed in a similar fashion to people.[8] Cross sectional imaging such as magnetic resonance imaging (MRI) is often performed for diagnosis, and management consists of symptomatic treatment of pain using nonsteroidal anti-inflammatory drugs or steroids, opioids, or other drugs targeted at pain and inflammation. Spinal surgery is also often performed for canine "patients" with neurologic deficits or refractory pain.[8]

The high prevalence of IVDD in certain breeds such as the dachshund provides a platform to expediently conduct preclinical studies that can be designed to adhere to CONSORT standards for human clinical trial design and reporting. For example, our institution alone manages approximately 200 cases per year of canine IVDD, most of which would be available for enrollment in treatment studies. Several
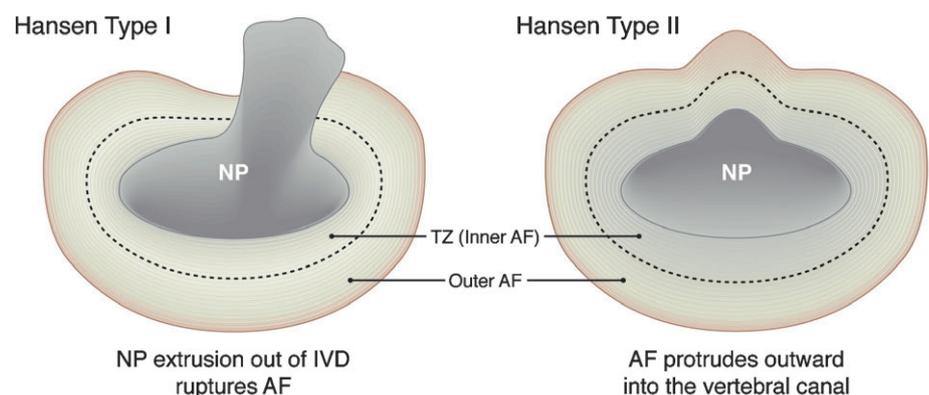

FIGURE 2 Herniation of the intervertebral disc (IVD): Hansen type I (left) and type II (right) IVDD with nucleus pulposus (NP) protrusion through and AF (annulus fibrosus) rupture in type I and protrusion of AF into the vertebral canal in type II. TZ, transitional zone

Hansen Type I

Hansen Type II

NP

NP

TZ (Inner AF)

Outer AF

NP extrusion out of IVD ruptures AF

AF protrudes outward into the vertebral canal

TABLE 2 Characterization models for the canine IVD

Research questions	Cells	Tissue	Organ	In vivo	Groups	Measurements	NCD	CD	Outcomes
			Experimental conditions						
Effects of hypoxia on NC organization[55]	X				Monolayer and 3D; hypoxia (3.5% O_2) and normoxia (21% O_2)	Histology; matrix production	X		Under hypoxia NCs organize themselves and produce matrix similar to in vivo; not in normoxia
Investigated Wnt/B-catenin signalling[56]	X	X			Healthy and early degeneration	Histology; B-catenin expression; qRT-PCR for T, KRT8, axin2, cyclin D and c-myc	X	X	Dual role of B-catenin in NC-rich progenitor cells and also in early disease
Gene expression profiling of early intervertebral disc degeneration[57]	X	X			NCs; mixed NC + CLC; CLCs	Histology; microarray; qRT-PCR for T, KRT8 and Wnt target genes; B-catenin and caveolin-1 expression	X	X	Early degeneration involves down-regulation of Wnt signaling and caveolin-1 expression—Essential to physiology and preservation of NCs
Osmolarity and clustering regulate NC phenotype[58]	X				(DMEM)/F12 (300 mOsm/L; a-MEM (300 mOsm/L); a-MEM (400 mOsm/L)	NC morphology and matrix (histology); qRT-PCR for T, KRT8 and 18 and matrix genes; DNA/GAG	X		Culturing NCs in native clusters and high osmolarity media retain NC phenotype
Proteomic and biomechanical chracterization[59]		X			NCD and CD	iTRAQ proteomics of secretome; western blot; histology/IHC; matrix and biomechanics	X	X	Differences in ECM proteins between species - decorin, biglycan, fibronectin, fibromodulin and HAPLN1; CD less stiff than NCD
Characterization of inflammatory profile in the healthy and degenerate canine IVD[39]		X			Healthy and degenerate	Levels of PGE2, cytokines, chemokines, and matrix components; histology and COX-2 expression	X	X	PGE2 and CCL2 levels in degenerated IVDs significantly higher than healthy IVDs; COX-2 increased with grade degeneration
Discectomy model of cervical disc degeneration[60]				X	Discectomy versus adjacent control IVD	Histology; MRI; radiographs		X	Discectomy induced degenerative changes; loss disc height, modic changes and sclerosis
Whole genome screening for skeletal dysplasia and disc degeneration[61]	Blood	X			Skeletal dysplasia within 1 breed; IVD degeneration across multiple breeds	GWAS; genotyping; qRT-PCR; semi-qRT-PCR	X	X	FGF4 retrogene on CFA12 responsible for chondrodystrophy and IVD degeneration
Inflammatory profile of herniated canine IVDs[62]		X			Herniated (H), affected nonherniated (NH) disc, and adjacent nonaffected (NA) disc; control discs	qRT-PCR and protein expression of inflammatory cytokines; neurological assessment?	?	?	Gene—IL-6 and TNFa up-regulation and IL-1b down-regulation with herniation; protein expression varied for IL-6 and associated with positive outcomes; infiltration of monocytes and macrophages

recent large-scale multicenter placebo controlled randomized veterinary studies have been performed using the canine spontaneous model of IVDD.[63,64] While these studies focused on neuroprotective strategies aimed at treating the 10%-15% of dogs with IVD that can develop severe neurologic complications rather than treatments targeted specifically at disc degeneration, they still serve as important proof of concept regarding feasibility of large-scale studies using this disease model.[54]

Studies in the dog model of IVDD can be performed using clinically relevant outcome measures shared across species including quality of life assessments, measures of neuropathic pain, and locomotor outcomes. Techniques such as owner-derived quality of life questionnaires, quantitative sensory testing (QST), various locomotor scoring systems and kinematic gait assessments have all been validated for use in dogs with IVDD.[65,66] Because these outcome measures mirror those of human trials, positive results may be more predictive of translational success. The utility of canine translation models for the study of chronic pain has recently gained attention in the literature; however, most canine pain studies to date have focused on osteoarthritis while the potentially valuable scenario of chronic disc-associated pain in dogs with IVD has been under-explored.[67–69]

TABLE 3 Therapeutic models for the canine IVD

Research model	Experimental conditions							Outcomes
	Cells	Tissue	Organ	In vivo	NCD	CD	Pain	
Therapeutic models								
Cell therapies								
Autologous disc chondrocyte transplantation[86]	X			X	?	?		Cells were viable and proliferative after transplantation; produced matrix & maintained disc height
Wharton's jelly cell transplantation[87]	X			X		X		Cells were viable after 24 weeks post transplantation into degeneration induced NP of beagles. WJC treated beagles had smaller disc reduction, well preserved structure and ACAN/COL2/SOX-9 gene upregulation compared to nontreated groups.
Adipose stem cells[88]	X			X	?	?	X	Autologous adipose tissue derived stem cells promoted disc regeneration; produced matrix and maintained disc height.
BMP2 and MSCs[89]	X				X	X		BMP2 showed regenerative effects on chondrocyte-like-cells with more healthy NP matrix vs TGF-β1. Adding MSCs to BMP2 treated cells did not show additional regenerative effects on either CD or NCD.
Chondrocyte transplantation[90]	X			X	?	?	X	Cells were viable and proliferative after transplantation, produced ECM and maintained disc height. All 3 pain scores showed significant reduction of low back pain.
Transplant activate NP cells[91]	X			X		X		Cryopreserved vs activated NP cells showed no difference in treatment of in-vivo canine models. Disc height was better maintained compared to nontreated groups, cells maintained viability and slowed disc degeneration.
Notochordal conditioned media								
Canine NCCM on bovine NP cells[92]	X				X	X		Difference in proteoglycan production was seen with different NCCM concentrations. However, there was no dose dependency of NCCM for cell proliferation. NC cells maintained phenotype in masses in suspension and in monolayer.
Canine NCCM contains CTGF and increases proteoglycan[93]	X				X	X		NC cells contain growth factor CTGF (upregulates aggrecan, versican, HAS-2 gene). Found no difference in CTGF gene expression in NCD vs CD canine NC cells. Study suggests CTGF as anabolic factor and dependent on population of NC cells in disc.
NCCM protects NP cells from degradation and apoptosis[94]	X				X			NC cell secreted factors prevent NP apoptosis via inhibition of caspase-9 and − 3/9. Degradation prevented via upregulation of anabolic and matrix protection genes.
Molecular therapy: NCCM characterization[95]	X			X	X			Found TGF-β and CTGF to be major hubs in protein interaction networks. Treatment with TGF-β1 and CTGF in vitro promoted ECM synthesis, increased cell proliferation and decreased cell death. Injection of TGF-β1 and CTGF in rat tail injury restored NP.
Canine NC conditioned media effects on arthritic chondrocytes[96]	X				X			NCCM restored cartilage matrix production of end-stage human OA chondrocytes and suppressed production of inflammatory mediators. NCCM was age and disease dependent based on human donors >55y.o
Canine NCCM[97]	X				X	X		NCCM increased NP cell proliferation, GAG production, and increased NP phenotypic gene expression. BMS cells showed increased GAG production in NCCM but no gene level effects and did not increase GAG content in NP cells compared to NCCM alone.
Canine NCCM[98]	X				X	X		NC cells did not maintain phenotype in culture of alginate beads. NC and NP cell coculture ECM content and anabolic gene expression showed no difference. MSCs and NC coculture showed increased GAG content and Brachuary T expression.
Bioactive ligands								
BMP7[99]	X			X	X			hBMP7 transfected NP cells injected into cryopreserved IVDs and implanted in dogs. Treated dogs maintained structural integrity of disc, ECM and biomechanical properties.
IL-10 and TGFb[100]	X					X		Treatment suppressed IL-1β and TNF-α and inflammatory responses.
BMP7[101]	X			X		X	X	rhBMP-7 treatment in vitro increased matrix production and gene expression of ACAN and COL2A1. However, no regenerative effects were observed for in vivo treatments at IVD. Extra-discal bone formation observed.
Caveolin and repair[102]	X				X	X		Caveolin-1-null mice had collagen rich ECM and fewer NCs with high apoptosis activity compared to wild-type mice. Found high caveolin-1 expression and cell dead in degenerate canine IVDs. Yet, caveolin-1 silencing decreased GAG content but rescued by caveolin-1

TABLE 3 (Continued)

| Research model | Experimental conditions | | | | | | | Outcomes |
	Cells	Tissue	Organ	In vivo	NCD	CD	Pain	
								scaffolding domain (CSD). CSD treatment also increased TGF-β/pSmad2 signaling.
Link-N[103]	X			X	X			Human and canine link-N showed species-specific effects on chondrocyte like cells but both induced negligible GAG deposition in canine CLCs.
Hydrogels								
Disc replacement cervical spine[104]	X	X		X		X		Disc height retention and physiological hydration, matrix production and integration into host tissue after 16 weeks. Still lacks mechanical properties compared to native tissue.
Poly(ε-caprolactone-co-lactide)-b-poly(ethylene glycol)-bpoly(ε-caprolactone-co-lactide) hydrogel + celecoxib[105]	X	X		X		X	X	No adverse reaction to hydrogel injection. 9/10 dogs showed back pain reduction, 3/10 dogs had recurring pain after 3 months.
Polyester amide microspheres[106]	X			X		X		No degenerative changes occurred post injection of PEAM compared to nontreatment groups, good cyto-compatibility in vitro.
Poly-N-isopropylacrylamide MgFe-layered double hydroxide hydrogel and celecoxib[107]	X	X		X		X	X	Good biocompatibility and safe application of hydrogel. However, controlled release of CXB had only limited in hibition of PGE2 and resulted in mild IVD degeneration.

QST describes a collection of techniques used commonly in human clinical studies of LBP to quantify pain, assess sensory abnormalities, and document treatment effects. The type of stimulus evaluated using QST varies, with mechanical, thermal, and vibrational stimuli reported for use in a variety of animal disease models and in people [70–72]. Several recent studies have reported the use of both mechanical QST (with an electronic von Frey anesthesiometer, von Frey filaments, or other devices) and thermal sensory testing to document sensory abnormalities in dogs with IVDD.[73,74] As mentioned above, these studies have also focused on the small percentage of dogs with IVD presenting with severe neurologic deficits; however, they demonstrate feasibility of QST using the canine IVDD model and suggest that translation of these protocols to dogs presenting only with back pain is likely possible. There are also several clinical metrology instruments (CMIs) that have been validated for use as owner-derived pain assessments in veterinary studies. The Canine Brief Pain Inventory (CBPI) and the Helsinki Chronic Pain Index were both developed specifically to evaluate chronic pain in dogs with osteoarthritis but have direct relevance to canine spinal pain.[75,76] Activity monitors similar to the Fitbit are available for dogs and have been validated for monitoring step counts, active minutes, and intensity of activity.[77–79] Various canine activity monitors have been used as surrogate markers of decreased mobility associated with chronic pain in dogs and lend themselves to outcomes assessment in clinical studies of canine IVDD.[80]
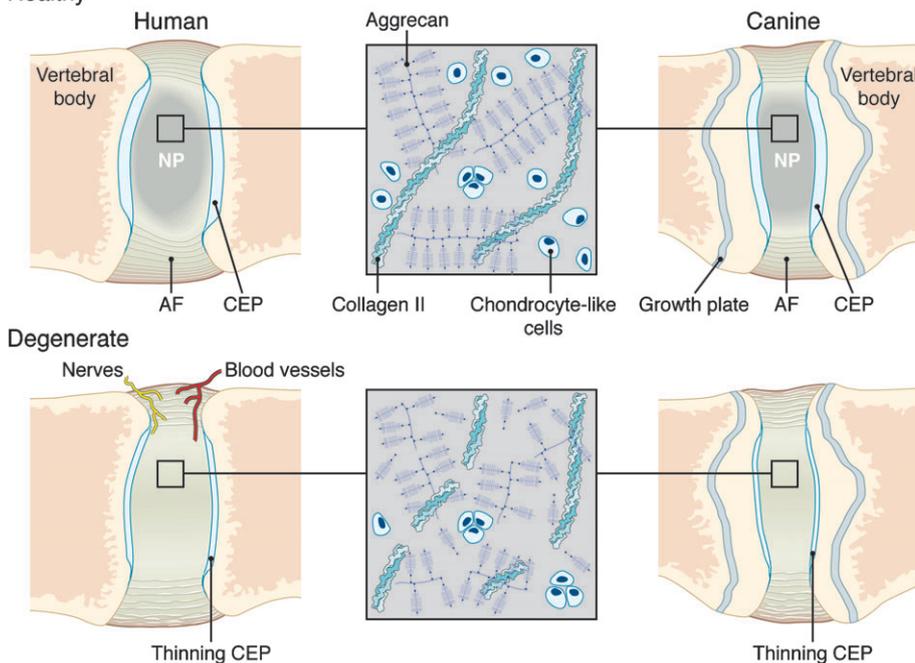

FIGURE 3 Comparison of the healthy and degenerate human (left) and canine intervertebral disc (IVD) (right) on the gross and molecular level with neurovascular ingrowth, decreased in chondrocyte-like cells and broken aggrecan and collagen II in the degenerate nucleus pulposus (NP)
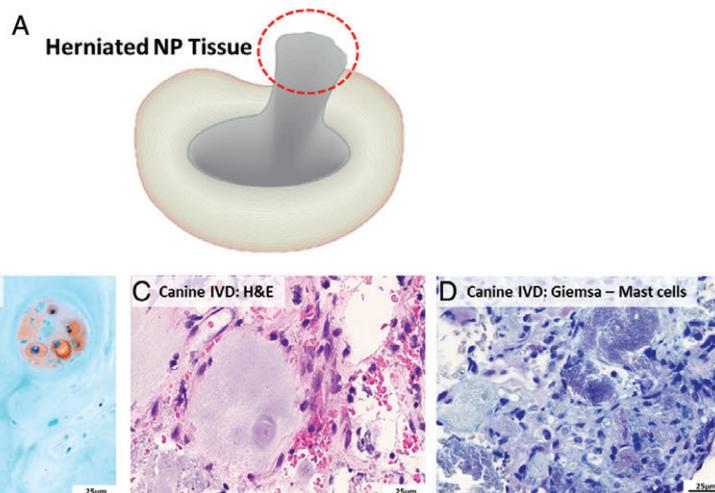
FIGURE 4 Cells within the degenerate canine intervertebral disc (IVD) where nucleus pulposus (NP) tissue was surgically removed from herniated canine IVDs (A-D); schematic of herniated IVD (a); safranin O fast staining of cell clusters in diseased disc (B) and hematoxylin and eosin staining of red blood cells in granulation tissue suggestive of angiogenesis (C); giemsa staining of mast cells (dark blue/purple, ×40) (D)

7 | CHALLENGES ASSOCIATED WITH THE CANINE MODEL

While the canine clinical model of IVDD may hold significant promise in enhancing translational efficiency, a few differences in canine and human vertebral column structure must be considered. Minor differences include number of vertebrae and overall size: the typical human vertebral column has 12 thoracic and 5 lumbar IVDs, while the canine has 13 thoracic and 7 lumbar IVD. Additionally, human CEPs are thicker than those of the dog due to expanded layers of chondrocytes.[81,82] This results in an increased number of CEP irregularities compared to those observed dogs.[11] The canine vertebral growth plates close at skeletal maturity (~8 months) whereas in people, secondary ossification centers (ring apophysis) develop during teenage years then close at skeletal maturity.[83] The canine vertebral column contains growth plates throughout, which are responsible for the majority of vertebral growth. This species difference is relevant for histologic and imaging-based grading of IVDD and CEP changes, but is unlikely to affect translational relevance.[18] Differences in spinal biomechanics between canine and human, namely the quadrupedal nature of the dog placing the spine at a horizontal nature vs the vertical human spine due to bipedalism, must also be considered. Recent investigations however, have demonstrated that the axial loading effects are similar across the vertebral column in both species, suggesting that biomechanical differences may be less relevant than might be expected.[11,29,84] Indeed, ligaments and muscle play a key role in the stabilization of the spine both in bipeds and quadrupeds.[85]

8 | EXPERIMENTAL STUDIES USING THE CANINE MODEL—BIOLOGICAL STRATEGIES AND TREATMENTS

Current available treatment options for both dogs and people experiencing IVDD are either largely conservative and aim to manage pain and discomfort, or highly invasive surgeries which do little or nothing to stop or prevent further degeneration and do not attempt disc restoration. Physiotherapy is often recommended to help both dogs and people build core strength and retain normal everyday movements through relief of compression on the IVDD affected areas.[8] Given the potential benefits as a translational model, several recent studies have utilized the canine model to investigate regenerative therapies for IVDD via cell therapies, notochordal conditioned media (NCCM), ligands (growth factors and gene therapy) and various hydrogels as summarized in Table 3.

Cell based therapies are a common means of targeting matrix regeneration of the IVD, including the use of autologous IVD cells, CLCs, bone marrow- and adipose-derived mesenchymal stem cells (MSCs), and differentiated cells. The studies in Table 3 have demonstrated the positive regenerative potential of CLCs via increases in cell viability, matrix production and disc height integrity; however, CLC treatment is limited in both human and canine patients based on the stage of IVDD and is most beneficial at earlier stages.[86,90,91] Recently, the safety and feasibility of autologous bone marrow-derived MSCs to treat disc degeneration was evaluated in the canine clinical model of IVDD, which demonstrated the safety of intradiscal injection of MSC.[108] Small animal experiments have demonstrated degeneration reduction in the progression of IVDD post treatment with MSCs, and additional studies in the beagle model have proven useful in inhibiting IVDD, with the potential for promoting continued avascularity.[109] Similarly, adipose derived stem cells enhance disc regeneration[88] along with Wharton's jelly cells (WJCs) which are capable of differentiating into NP cells in coculture.[87]

Significantly, there are a number of studies using NCCM on canine IVD cells, which have shown to decrease apoptosis of NP cells, increase cell proliferation, slow degradation of ECM and promote ECM synthesis.[94,95,97,98,110] Additionally, several growth factors from notochordal cells have been identified, including TGF-β1 and CTGF that aid in matrix production.[89,92,95] Growth factor treatments such as GDFs/BMPs including caveolin also promote matrix biosynthesis resulting in regeneration of healthy disc tissue.[89,102,111] Furthermore, with regards to gene therapy, exploration of the immune and inflammatory responses and pathways that follow can function as targets to slow the degenerative process of the IVD.[100,103]

Within the field of tissue engineering, several cell seeded hydrogels have been proposed to treat IVDD, which include replacement of the entire or part of the disc in in vivo or mainly for use as drug control release systems. Some examples are biocompatible hydrogels

such as PCLA-PEG-PCLA (poly(ε-caprolactone-co-lactide)-b-poly(ethylene glycol)-bpoly(ε-caprolactone-co-lactide)), agarose, and polyester amides.[104–106] Additionally, hydrogels have also been used to encapsulate drugs for delivery into the IVD.[105–107]

These various therapies, as summarized, demonstrate the potential of the CD dog model as a clinically relevant model to evaluate regenerative IVDD therapies which can later translate to human conditions. However, unexplored is the potential of using organ culture as a tool to investigate and screen biological therapies for IVDD.

9 | OPPORTUNITIES FOR FUTURE EXPLORATION

When critically evaluating canine spontaneous IVDD for suitability as an animal model of IVDD, what remains largely unknown is the contribution of immune and inflammatory responses to the disease process in dogs. Degeneration of the human IVD has been shown to trigger responses that elicit an infiltration of mast cells (MCs) and macrophages (MØs) into the disc, particularly in injured areas and regions of granulation.[112] MCs are involved in the body's first responses to injury, whereby they release granules of bioactive ligands to stimulate healing and repair.[112,113] Likewise, when propagated by injury, MØs release cytokines such as TNF-α, IL-1β, and prostaglandins.[39] These cytokines upregulate catabolic processes that breakdown IVD matrix and limit regenerative processes. The specific roles of MCs and MØs in the pathogenesis of IVDD in people and dogs, however, remain to be elucidated. Coupled with these responses are pain predictors such as substance P and NGF, which can facilitate nerve ingrowth and pain experienced by canine and human patients with IVDD.[31,114] Further studies are required to better understand whether the inflammatory responses observed in human IVDD are modeled by the canine clinical disease.

10 | ARE CHONDRODYSTROPHIC DOGS A GOOD TRANSLATIONAL MODEL FOR HUMAN IVDD?

This review summarizes and evaluates the criteria necessary for qualifying animal models of IVDD for use in translational application to the human condition, as is visible in Table 1. As summarized in Figure 3, the biochemical and cellular composition of the IVD is similar across the CD canine and human species. Aside from thinner CEPs and smaller overall size of the canine IVD, the structures of the healthy discs are very similar. Using histological stains as a comparison, cell clusters and angiogenesis along with the infiltration of MCs are similar pathologies that also occur in the human degenerate IVD (Figure 4).[115] While further studies are required to investigate the presence of neurovascular ingrowth, immune infiltration and mechanisms underlying IVD-associated pain in the canine degenerate IVD as occurs in the human, other characteristics of human IVDD are commonly present in the CD canine model. Additionally, the similarities in this clinical population as well as diagnostic and treatment

methods demonstrate the potential suitability of CD canine IVDD as a model for human IVDD.

11 | CONCLUSION

A number of experimental animal models exist that attempt to recapitulate IVDD in people. Available models are limited by notochordal cell populations, small size, and artificial induction of the disease that do not mimic spontaneous mechanisms of degeneration. This review highlights aspects of the spontaneous canine clinical model of IVDD that make it attractive as a preclinical model for translational studies. Future work should focus on defining the inflammatory and symptomatic profiles associated with painful IVDD to allow for the better understanding of how these relate to those observed in human IVDD.

Author contributions

K.A.T., S.A.M., S.T., M.G.W., and D.P. contributed to drafting the article or revising it critically for important intellectual content and final approval of the version to be submitted.

Conflict of interest

The authors have no conflicts to declare.

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